**UW Medicine - Pathology**

400-11-01-17

**Hybridization and Washing of Agilent 4x180k Array**

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| Adopted Date: Oct. 12, 2012Review Date: Oct. 21, 2012Revision Date: Oct. 21, 2012Under Revision: |

PURPOSE

To co-hybridize labeled gDNA of the test sample and the normal control reference sample onto the microarray chip slide and wash before scanning the slides.

PROCEDURE

### Material and Equipment

1. Water both to 37°C
2. Heat block capable of temperatures to 98°C
3. Microcentrifuge (12,000 x g capability)
4. Mini centrifuge
5. Vortex mixer
6. Hybridization Chamber, stainless
7. Hybridization oven; set at 65°C
8. Hybridization oven rotator
9. Pipettmen p10, p20, p200, p1000
10. Aerosol-resistant sterile pipette tips
11. Sterile microcentrifuge tubes, 1.5 ml, 0.2 ml
12. Ice buckets
13. 4x180K array slide (Agilent G4449A) or 2 x 400K array (Agilent G4507A)
14. Gasket slide (Agilent Cat#G2534-60015)

### Reagents

1. PBS Human Cot-1 DNA (Invitrogen Cat#15279-011)
2. Agilent hybridization kit (Agilent Cat#5188-5200)

### Procedures

1. **Preparation of Labeled gDNA for Hybridization**
2. Prepare the 10× Blocking Agent:
3. Add 1,350 μl of DNase/RNase-free distilled water to the vial containing???
4. Lyophilized 10× aCGH Blocking Agent (included in the Oligo aCGH/ChIP-on-chip Hybridization Kit).
5. Leave at room temperature for 60 minutes and mix on a vortex mixer to reconstitute sample before use or storage.

***Note***: The 10× Blocking Agent can be prepared in advance and stored at -20°C. Leave at room temperature for 60 minutes and mix on a vortex mixer to reconstitute sample before use or storage.

1. Preparation of samples for Hybridization
2. Equilibrate water baths or heat blocks to 95°C and 37°C or use a thermal cycler.
3. Mix the components according to the microarray format to prepare the
4. Prepare the hybridization Master Mix
	1. Equilibrate water baths or heat blocks to 95°C and 37°C or use a thermal cycler.
	2. Mix the components according to the microarray format to prepare the Hybridization Master Mix (**Table 8** or **Table 9**).

**Table 8.** Hybridization Master Mix for 2x array

|  |  |  |  |
| --- | --- | --- | --- |
| **Component** | **Volume (μl) per hybridization** | **× 2 rxns (μl) (including excess)** | **Note** |
| Cot-1 DNA (1.0 mg/mL)\* | 25 | 60 |  |
| 10× aCGH Blocking Agent† | 26 | 62.4 |  |
| 2× HI-RPM Hybridization Buffer† | 130 | 312 |  |
| **Total**  | 181 | 434.4 | Divide 181 l for each pair |

\* Use Cot-1 DNA (1.0 mg/mL) from the appropriate species.

† Included in the Oligo aCGH/ChIP-on-chip Hybridization Kit

**Table 9.** Hybridization Master Mix for 4x array

|  |  |  |  |
| --- | --- | --- | --- |
| **Component** | **Volume (μl) per hybridization** | **× 4 rxns (μl) (including excess)** | **Note** |
| Cot-1 DNA (1.0 mg/mL)\* | 5 | 24 |  |
| 10× aCGH Blocking Agent† | 11 | 52.7 |  |
| 2× HI-RPM Hybridization Buffer† | 55 | 264 |  |
| **Total**  | 71 | 340.7 | Divide 71 l for each pair |

\* Use Cot-1 DNA (1.0 mg/mL) from the appropriate species.

† Included in the Oligo aCGH/ChIP-on-chip Hybridization Kit

* 1. Add the 71 μl of the Hybridization Master Mix each tube (final volume is 110 l).

 **Table 10.** Volume of Hybridization Master Mix per hybridization

|  |  |  |  |
| --- | --- | --- | --- |
| **Microarray format** | **Volume of Hybridization****Master Mix** | **Total volume** | **Loading for hybridization** |
| 2x array | 181 μL | 260 μL | 245 μL  |
| 4x array | 71 μL | 110 μL | 100 μL |

* 1. Mix the sample by pipetting up and down, then quickly spin in a centrifuge to drive contents to the bottom of the reaction tube.
	2. Transfer sample tubes to a circulating water bath or heat block at 95°C. Incubate at 95°C for 3 minutes, then immediately transfer sample tubes to a circulating water bath or heat block at 37°C. Incubate at 37°C for 30 minutes (**Table 11**).

 **Table 11.** Thermal cycler program

|  |  |  |
| --- | --- | --- |
| **Step** | **Temperature** | **Time** |
| Step 1 | 95°C | 3 minutes exactly |
| Step 2 | 37°C | 30 minutes |

* 1. Remove sample tubes from the water bath, heat block, or thermal cycler. Spin 1 minute at 6000 × g in a centrifuge to collect the sample at the bottom of the tube.
	2. The samples are ready to be hybridized.
1. **Hybridization Assembly**
2. Load a clean gasket slide into the Agilent SureHyb chamber base with the gasket label facing up and aligned with the rectangular section of the chamber base. Ensure that the gasket slide is flush with the chamber base and is not ajar.
3. Slowly dispense hybridization sample mixture onto the gasket well in a “drag and dispense” manner: 100 μl (for 4x microarray) or 245 μl (for 2x microarray).

***Note***: make sure the orientation of the slide.

1. For multi-pack microarray formats load all gasket wells before you load the microarray slide. For multi-pack formats, refer to “Agilent Microarray Layout and Orientation” on page 109.

***Note*:** Keep the temperature of hybridization sample mixtures as close to 37°C as possible. To do this, process them in small batches and/or put them on a heat block, thermal cycler or in an oven.

1. Put a microarray slide “active side” down onto the gasket slide, so the numeric barcode side is facing up and the “Agilent”-labeled barcode is facing down. Assess that the sandwich-pair is properly aligned.

**Note:** for multi-pack microarray formate (i.e. 2x and 4x array), load all gasket wells before you load the microarray slides. Fore multi-pack formats, refer to “Agilent microarray layout and orientation”.

1. Put the SureHyb chamber cover onto the sandwiched slides and slide the clamp assembly onto both pieces.
2. Hand-tighten the clamp firmly onto the chamber.
3. Vertically rotate the assembled chamber to wet the slides and assess the mobility of the bubbles. Tap the assembly on a hard surface if necessary to move stationary bubbles.
4. **Hybridize on Oven**
5. Put assembled slide chamber in the rotator rack in a hybridization oven set to 65°C. Set your hybridization rotator to rotate at 20 rpm.
6. Hybridize at 65°C for 24 hours (for 4x microarrays) or for 40 hours (for 2x microarray)

***Note*:** If you are not loading all the available positions on the hybridization rotator rack, be sure to *balance* the loaded hybridization chambers on the rack similar to a centrifuge to prevent unnecessary strain on the oven motor.

* 1. **Reference**

Agilent Arrays-based CGH for genomic DNA analysis protocol version 7.1 p. 63-69.

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Technologist: Yuhua Liu and Yang Zhou Yajuan Liu PhD, FACMG

**UW Medicine - Pathology**

**Cytogenetics and Genomics**

**SIGNATURE PAGE FOR POLICIES AND PROCEDURES**

Procedure / Policy Title: **Hybridization and Washing of Agilent 4x180k Array**

Procedure / Policy Number: 400-11-01-17

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| --- | --- | --- |
| **STAFF NAME**: (printed) | **STAFF SIGNATURE** | **DATE REVIEWED** |
| Chen, Xiaoqin |  |  |
| Darrin, Delores |  |  |
| DeHoogh-Grigsby, Debi |  |  |
| Donovan, Chris |  |  |
| Kraus, Jean |  |  |
| Liu, Yuhua |  |  |
| McInnis, Donna |  |  |
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| Whalen, Sara |  |  |
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